

The Acetylcholine Receptor as a Cellular Receptor for Rabies Virus

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Characterization of specific host cell receptors for enveloped viruses is a difficult problem because many enveloped viruses bind to a variety of substrates which are not obviously related to tissue tropisms in the intact host. Viruses with a limited cellular tropism in infected animals present useful models for studying the mechanisms by which virus attachment regulates the disease process. Rabies virus is a rhabdovirus which exhibits a marked neuronotropism in infected animals. Limited data suggest that spread occurs by transsynaptic transfer of virus. The results of recent experiments at Yale suggest that viral antigen is localized very soon after injection at neuromuscular junctions, the motor nerve endings on muscle tissue. On cultured muscle cells, similar co-localization with the acetylcholine receptor is seen both before and after virus multiplication. Pretreatment of these cells with some ligands of the acetylcholine receptor results in reduced viral infection. These findings suggest that a neurotransmitter receptor or a closely associated molecule may serve as a specific host cell receptor for rabies virus and thus may be responsible for the tissue tropism exhibited by this virus. In addition to clarifying aspects of rabies virus pathogenesis, these studies have broad implications regarding the mechanism by which other viruses or viral immunizations might mediate autoimmune diseases such as myasthenia gravis.

INTRODUCTION

Rabies virus belongs to the rhabdovirus family, *Lyssavirus* genus, which are enveloped, negative-stranded RNA viruses with a distinctive morphology resembling a bullet (for review, see [1,2]). The disease is characterized by extensive involvement of the central nervous system and almost always ends in a fatal outcome after the onset of clinical signs or symptoms. The pathogenesis of rabies virus infection with its restricted spread to defined cell types, especially neurons, argues for the existence of highly specific host cell receptors. In this brief review, we summarize current literature regarding host cell receptors for enveloped viruses and the role of these receptors in the pathogenesis of virus infections. In addition, we discuss the role of attachment in governing the pathogenesis of rabies virus infection and propose sites of rabies virus entry into the peripheral nervous system. An hypothesis defining the identity of a highly specific host cell receptor is reviewed. The relevance of experimental findings with rabies virus to the study of other neurologic disorders is presented.

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INTERACTION OF VIRUSES WITH CELL SURFACE RECEPTORS

The first step in the interaction of viruses and cells is the attachment of the virus to the cell surface (for review, see [3]). In the virus-host cell infectious cycle, attachment is followed by entry of the virus into the cell, uncoating of the nucleic acid, transcription and translation directing synthesis of viral proteins, replication of viral nucleic acid, assembly of virus, and release of virus particles from the cell. Attachment of the virus to the cell surface is mediated by specific components on the surface of the virus which interact with complementary molecules on the cell surface. There is considerable evidence that the specific attachment components of enveloped viruses are glycoproteins. Enveloped viruses, including rabies virus, are usually covered with glycosylated proteins, forming spikes extending from a lipid bilayer membrane. Removal of the virus spike glycoprotein enzymatically or genetically renders some negative-stranded viruses noninfectious [4,5]. For several enveloped viruses, the glycoproteins involved in attachment of the virus to some cell surfaces have been identified [6,7,8].

In general, less is known about the molecular structure of the host cell receptors for viruses (reviewed in [9,10]). The plasma membrane of the cell is a lipid bilayer composed of phospholipids, cholesterol, glycolipids, and gangliosides. Embedded within and spanning the lipid bilayer are integral membrane glycoproteins with the terminal glycosylated segment of the polypeptide chain facing the outside. In addition, more weakly associated with the membrane are peripheral or extrinsic membrane proteins. Membrane proteins accessible to the exterior include regulatory molecules, enzymes, ion channels, recognition sites for other cells, and receptors for hormones, neurotransmitters, and immunoglobulins [9]. It is likely that some of these normal surface components serving useful cellular functions have been usurped and used as receptors by viruses [2]. The specificity, number, and distribution of these cellular receptors has been hypothesized to be responsible for the species specificity and tissue tropisms of viruses [11,12]. Thus, identification of the cellular receptors for viruses could contribute considerably to understanding the initial stages of viral infection and the relationship of this step in viral replication to the pathogenesis of viral diseases.

Although the precise functional identity of the molecules on cell surfaces serving as receptors for most viruses is unknown, the receptors are often sensitive to proteolytic enzymes, demonstrating that they are proteins or glycoproteins [13,14,15]. In some cases, the host cell receptor is essential for the constitutive functioning of the cell, since the receptors cannot be cleaved by proteolytic enzymes without killing the cell [15]. Neuraminic acid or its derivatives (sialic acids) have been identified as attachment determinants for many myxoviruses (influenza) and paramyxoviruses (parainfluenza, mumps) [6,8,16]. The binding site for Epstein-Barr virus on lymphoid cells is closely associated with but not identical to complement (C3d) receptors [17]. Finally, Semliki Forest virus spike proteins may bind to human HLA-A and HLA-B and murine H2K and H2D histocompatibility antigens [18], although it is uncertain that these antigens are specific receptors [19]. Thus, any molecule associated with the cell surface represents a potential virus receptor if it possesses the ability to interact with components on the surface of the virus.

However, enveloped viruses may bind to many cell types and inanimate surfaces, such as glass or nitrocellulose, which are not obviously related to tissue tropism. A molecule can be considered a specific receptor if its interaction with the virus is followed by internalization and infection [3,10]. Difficulties in defining specificity

of receptors have been discussed elsewhere [3,20]. The concept of specific receptors is helpful but not entirely accurate, since binding to red blood cells, for example, is an important pathogenic mechanism contributing to spread of some viruses although the virus may not replicate in these cells. In addition, binding of virus to cell receptors is only the first in a series of steps determining specific cell vulnerability and does not always guarantee successful viral production [21]. For example, many molecules after binding to their specific receptors on the cell surface are internalized within coated vesicles, a process known as receptor-mediated or absorptive endocytosis. It has been demonstrated that Semliki Forest virus, an enveloped virus of the Togavirus family, binds to the cell surface and enters by absorptive endocytosis [22]. Defects in internalization after binding can lead to a failure in replication.

Specificity of attachment may reside in the particular amino acid sequence of a polypeptide or in a carbohydrate side chain occupying only a portion of a large molecule. In the case of rabies virus, a single amino acid substitution replacing arginine at position 333 of the glycoprotein molecule renders the virus non-pathogenic [23]. Specific amino acid sequences on the host cell may similarly govern attachment. These determinants may be shared by different molecules on a variety of cell types and their distribution may establish the host cell specificity for a particular virus.

SITES OF ATTACHMENT AND SPREAD OF RABIES VIRUS

Rabies virus is usually transmitted from animal to animal by inoculation as a result of bites (see [24] for a review of the pathogenesis of rabies). The incubation period for rabies is often variable and during this period viral replication in striated muscle has been observed [25,26,27]. It has been suggested that initial infection of muscle might be crucial in amplifying the inoculum virus which subsequently enters the peripheral nervous system. Forty-eight hours after intramuscular inoculation of rabies virus into infant rodents, viral antigen has been localized by immunofluorescence in neuromuscular and neurotendinal spindles [24,28]. These sensory stretch receptor organs were postulated to represent the probable deep sites of viral entry into the nervous system, although virus particles could be observed at motor nerve terminals (neuromuscular junctions) by electron microscopy [29]. Other evidence indicates that rabies enters the nervous system through the motor nerve terminal [27]. Rabies virus antigen could be detected on muscle cells of mice at sites similar in form and distribution to cholinesterase-positive sites (neuromuscular junctions) one hour after inoculation. A similar distribution of labeled virus could be detected by autoradiography six hours after infection. These findings demonstrate that rabies virus is present at the neuromuscular junction very shortly after inoculation of virus.

Upon entry of the virus into the peripheral nervous system, it spreads to the central nervous system at the level of the spinal cord. This process may be achieved by retrograde transport within axons since disruption of axons or blockade of axoplasmic flow prevents centripetal spread of virus [30,31]. Rabies virus has been observed in sensory dorsal root ganglia 60 hours after injection of virus into the foot pad [28]. It has also been detected in motor neurons in the ventral horn of the spinal cord at 20 hours post-inoculation and before involvement of dorsal root ganglia, providing further evidence for the primary uptake of virus at motor nerve endings [27]. After virus reaches the spinal cord, its ascent to the brain and spread

throughout the central nervous system is rapid. There is evidence that virus is disseminated throughout the central nervous system by direct transfer of virus from neuron to neuron at synapses. Virus particles have been observed by electron microscopy budding from the postsynaptic and adjacent membranes of dendrites and, to a lesser extent, from the plasma membrane of the perikaryon [26]. Budding particles or particles free in the intracellular space are taken up by adjacent presynaptic axon terminals by a process of endocytosis (Fig. 1). Although the virus has a widespread distribution throughout the nervous system, early infection is highly selective for certain neuronal populations [32]. For example, there is extensive localization of virus in rhinencephalic structures, including hippocampus and septal nuclei, with relative sparing of the neocortex. In the cerebellum, Purkinje cells are infected, while neurons in the adjacent molecular and granular layers are not. In rat brain, the binding of quinuclidinyl benzylate, an antagonist of the muscarinic AChR, is markedly decreased with the onset of symptoms [33]. After central nervous system infection, virus spreads centrifugally through nerves and infects the neurons of a number of parenchymal organs [34]. In the salivary glands, virus replicates in the acinar cells and buds from the apical surfaces of cells into the duct system [35], thereby gaining access to the exterior in saliva.

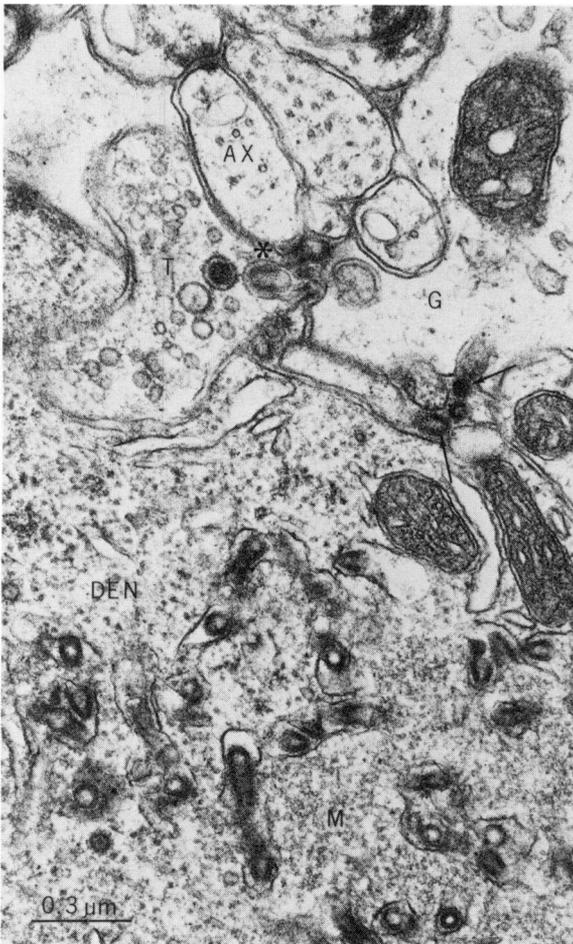


FIG. 1. Rabies virus replication and transfer in the molecular layer of the cerebellum of the mouse. Numerous rod-shaped virus particles are present within the apical dendrite (DEN) of a Purkinje cell. Dendrites, containing ribosomes and Nissl substance, are capable of synthesizing viral proteins and supporting replication. The particles are enclosed within membranous cisternae. Between the membranes in the cytoplasm are masses of granular matrix (M) material composed of viral nucleocapsid. Processes of glial cells (G), unmyelinated axons (AX), and axon terminals (T) occur adjacent to the dendrite. Some virus particles (arrows) are present in the extracellular space surrounding the cellular elements. A virus particle (*) lies within an invagination of the surface of a nerve terminal identified by its content of small synaptic vesicles. Such an image may represent a stage in the incorporation of a particle into the terminal by a process of endocytosis. $\times 44,000$.

THE ACETYLCHOLINE RECEPTOR AS A RABIES VIRUS RECEPTOR

The early discrete localization of rabies virus at the neuromuscular junction [27] suggests that some component of this region could function as a rabies virus receptor. Studies undertaken recently to identify the nature of the receptor indicate the receptor may be the acetylcholine receptor (AChR) [36]. The AChR [37] is an integral transmembrane protein which transduces a chemical signal (acetylcholine) released by the presynaptic nerve terminal into an electrical event consisting of a local depolarization of the postsynaptic membrane on which the receptor is located. It consists of four subunits which interact to form a monomer with a molecular weight of about 250,000 and dimensions of about 90×100 Å (Fig. 2). At the neuromuscular junction, the AChR is packed in high density on the junctional folds of the muscle fiber membrane.

In order to determine the receptor sites for rabies virus binding in the peripheral nervous system, mouse diaphragms with attached phrenic nerves were immersed in a suspension of rabies virus and stained with fluorescein-conjugated antibody to rabies virus. Virus antigen was localized at neuromuscular junctions after 30 minutes of exposure to virus and within peripheral nerves after four hours. Rabies virus binding and replication were also studied in cultured chick myotubes. Cultured myotubes (immature skeletal muscle cells) contain AChR within their membranes in both a diffuse low-density and a clustered high-density distribution [38]. When cultured myotubes were exposed to rabies virus, antigen was distributed in patches on the cell surface in a pattern similar to that observed following staining with rhodamine-labeled α -bungarotoxin. The latter is a polypeptide isolated from elapid snake venoms which binds specifically and nearly irreversibly to the nicotinic AChR of the fish electric organ and skeletal muscle [39]. Electron microscopy of infected myotubes showed association of rabies virus with specialized surface patches previously shown to contain a high density of AChR [40]. Finally, pre-treatment of myotubes with α -bungarotoxin and another ligand for the AChR, d-tubocurarine, dramatically reduced the number of myotubes that became infected. Both of these ligands bind to the 40,000 dalton α -subunit which contains the acetylcholine binding site of the AChR.

The results described above indicate that there are specific high-affinity host cell receptor sites for rabies virus at the neuromuscular junction and on myotubes and suggest that the receptor may be the AChR or a molecule very closely associated with it. Other molecules present along with AChR at the neuromuscular junction include acetylcholinesterase, laminin, fibronectin, and collagen type IV [41]. These proteins could act as virus receptor sites at the neuromuscular junction or on other cells, although the blocking effect of the cholinergic ligands becomes more difficult

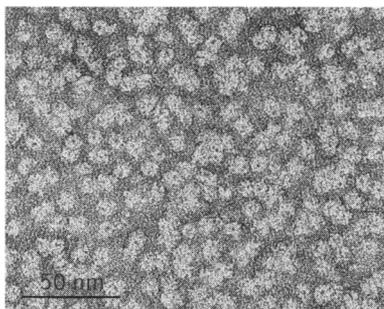


FIG. 2. Purified acetylcholine receptors from the electric organ of *Torpedo californica* negatively stained with 2 percent uranyl acetate. The receptor monomers appear as rosettes about 90 Å in diameter. Each rosette possesses a central pit 15–20 Å in diameter. $\times 270,000$.

to explain. However, it is conceivable that these inhibitors may interfere with one of a series of events taking place during binding and internalization of the virus.

If the AChR represents the rabies virus receptor, certain aspects of the pathogenesis of rabies may be more readily explained. The highest density of AChR occurs at the tips of the junctional folds of the neuromuscular junction (about 30,000 α -bungarotoxin sites/ μm^2 , [42]). Thus, binding of virus to the AChR may effectively enhance the probability of virus gaining access to the central nervous system through motor nerve terminals. Similarly, binding to AChR at central synapses may be responsible for the transfer and spread of virus from neuron to neuron by concentrating virus at postsynaptic sites in proximity to presynaptic axon terminals. Identification of a virus receptor should also have practical significance in providing a basis for preventing infection by blocking the attachment step. In the case of a disease such as rabies, this could be particularly useful because host defense mechanisms fail to prevent disease. Delay of infection, however, could increase the effectiveness of the normal immune response or treatment by active or passive immunization. Finally, there are a number of chronic neurologic diseases such as myasthenia gravis, multiple sclerosis, parkinsonism, chronic focal epilepsy, subacute sclerosing panencephalitis, and Alzheimer's disease for which some evidence of viral etiology exists [43]. One explanation for these diseases is that viral binding to a cellular constituent acting as a receptor alters the receptor in some way so that an autoimmune response is directed against it [20]. Identification of the specific neuronal constituents to which neurotropic viruses bind will allow an analysis of the potential effects of these interactions on functional or antigenic alterations of receptors.

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